

What is Claimed is:

1. A method of packaging a recombinant viral vector, comprising the steps of:

(a) providing a packaging cell, said packaging cell containing and expressing a nucleic acid encoding a mutant adenovirus E4orf6 protein, said E4orf6 protein containing
5 at least one mutation in the region encoding amino acids 230 to 260;

(b) transfecting or infecting said packaging cell with a nucleic acid that encodes a recombinant viral vector selected from the group consisting of adenovirus vectors and adeno-associated virus vectors, wherein said vector lacks a functional gene encoding E4orf6 protein;

10 and wherein said mutation renders said mutant adenovirus E4orf6 protein non-toxic to said transfected cells;

(c) culturing said transfected cells under conditions that permit expression of the mutant E4orf6 protein and the production of packaged recombinant viral vector therein; and then

15 (d) collecting packaged recombinant viral vector from said cultured cells.

2. The method according to claim 1, wherein said packaging cell is a mammalian cell.

20 3. The method according to claim 1, wherein said packaging cell is a 293 human embryonic kidney cell.

4. The method according to claim 1, wherein said mutation disrupts the interaction of the E4orf6 protein and the E1B-55kDa protein in said host cell.

25 5. The method according to claim 1, wherein said at least one mutation is in the region encoding amino acids 239 to 254.

30 6. The method according to claim 1, wherein said packaging cell is transiently transfected with said nucleic acid encoding said mutant adenovirus E4orf6 protein.

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7. The method according to claim 1, wherein said packaging cell is stably transfected with said nucleic acid encoding said mutant adenovirus E4orf6 protein.

8. The method according to claim 1, wherein said nucleic acid encoding said mutant adenovirus E4orf6 gene is carried by a plasmids, bacteriophage, cosmid or retrovirus.

9. The method according to claim 1, wherein said at least one mutation comprises a substitution mutation at position 240, 241, 243, 244, 248, or 251.

10. The method according to claim 9, in which said at least one substitution mutation is a substitution of arginine for an amino acid selected from the group consisting of glutamic acid, aspartic acid, serine, threonine, alanine and glutamine.

11. The method according to claim 1, wherein said nucleic acid encoding said mutant adenovirus E4orf6 protein encodes (i) an arginine 241 to glutamic acid substitution mutation, (ii) an arginine 243 to glutamic acid substitution mutation, or (iii) both an arginine 241 to glutamic acid substitution mutation and an arginine 243 to glutamic acid substitution mutation.

12. The method according to claim 1, wherein said nucleic acid encoding said mutant adenovirus E4orf6 protein encodes (i) an arginine 240 to glutamic acid substitution mutation, (ii) an arginine 251 to glutamic acid substitution mutation, or (iii) both an arginine 240 to glutamic acid substitution mutation and an arginine 251 to glutamic acid substitution mutation.

13. The method according to claim 1, wherein said viral vector is an adenovirus vector.

14. The method according to claim 1, wherein said viral vector is an adeno-associated virus vector.

15. A packaging cell, said packaging cell containing and expressing a nucleic acid encoding a mutant adenovirus E4orf6 protein, said E4orf protein containing at least one mutation in the region encoding amino acids 230 to 260 that renders said protein non-toxic to a host cell in which said protein is expressed.

16. The packaging cell according to claim 15, wherein said at least one mutation disrupts the interaction of the E4orf6 protein with the E1B-55kDa protein in a host cell.

17. The packaging cell according to claim 15, wherein said at least one mutation is in the region encoding amino acids 239 to 254.

19. The packaging cell to claim 15, wherein said packaging cell is a mammalian cell.

20. The packaging cell according to claim 15, wherein said packaging cell is a 293 human embryonic kidney cell.

21. The packaging cell according to claim 15, wherein said packaging cell is transiently transfected with said nucleic acid encoding said mutant adenovirus E4orf6 protein.

22. The packaging cell according to claim 15, wherein said packaging cell is stably transfected with said nucleic acid encoding said mutant adenovirus E4orf6 protein..

23. The packaging cell according to claim 15, wherein said nucleic acid encoding said mutant adenovirus E4orf6 protein is carried by a plasmid, bacteriophage, cosmid or retrovirus.

24. The packaging cell according to claim 15, wherein said at least one mutation comprises a substitution mutation at position 240, 241, 243, 244, 248, or 251.

25. The packaging cell according to claim 24, in which said at least one substitution mutation is a substitution of arginine for an amino acid selected from the group consisting of glutamic acid, aspartic acid, serine, threonine, alanine and glutamine.

26. The packaging cell according to claim 15, wherein said nucleic acid encoding said mutant adenovirus E4orf6 gene encodes (i) an arginine 241 to glutamic acid substitution mutation, (ii) an arginine 243 to glutamic acid substitution mutation, or (iii) both an arginine 241 to glutamic acid substitution mutation and an arginine 243 to glutamic acid substitution mutation.

27. The packaging cell according to claim 15, wherein said nucleic acid encoding said mutant adenovirus E4orf6 gene encodes (i) an arginine 240 to glutamic acid substitution mutation, (ii) an arginine 251 to glutamic acid substitution mutation, or (iii) both an arginine 240 to glutamic acid substitution mutation and an arginine 251 to glutamic acid substitution mutation.

28. A nucleic acid encoding a mutant adenovirus E4orf6 protein, said E4orf6 protein containing at least one mutation in the region encoding amino acids 230 to 260 that renders said protein non-toxic to a host cell in which said protein is expressed.

29. The nucleic acid according to claim 28, in which said at least one mutation disrupts the interaction of the E4orf6 protein with the E1B-55kDa protein in a host cell.

30. The nucleic acid according to claim 28, wherein said nucleic acid is a DNA.

31. The nucleic acid according to claim 28, wherein said nucleic acid is a plasmid, bacteriophage, plasmid or retrovirus.

32. The nucleic acid according to claim 28, wherein said at least one mutation comprises a substitution mutation at position 240, 241, 243, 244, 248, or 251.

33. The nucleic acid according to claim 32, in which said at least one substitution mutation is a substitution of arginine for an amino acid selected from the group consisting of glutamic acid, aspartic acid, serine, threonine, alanine and glutamine.

34. The nucleic acid according to claim 32, wherein said nucleic acid encodes (i) an arginine 241 to glutamic acid substitution mutation, (ii) an arginine 243 to glutamic acid substitution mutation, or (iii) both an arginine 241 to glutamic acid substitution mutation and an arginine 243 to glutamic acid substitution mutation.

35. The nucleic acid according to claim 32, wherein said nucleic acid encodes (i) an arginine 240 to glutamic acid substitution mutation, (ii) an arginine 251 to glutamic acid substitution mutation, or (iii) both an arginine 240 to glutamic acid substitution mutation and an arginine 251 to glutamic acid substitution mutation.

36. A mutant adenovirus E4orf6 protein, said E4orf6 protein containing at least one mutation in the region encoding amino acids 230 to 260 that renders said protein non-toxic to a host cell in which said protein is expressed.

37. The protein according to claim 36, wherein said at least one mutation is in the region encoding amino acids 239 to 254.

38. The protein according to claim 36, wherein said at least one mutation disrupts the interaction of the E4orf6 protein with the E1B-55kDa protein in a host cell.

39. The protein according to claim 36, wherein said at least one mutation comprises a substitution mutation at position 240, 241, 243, 244, 248, or 251.

40. The protein according to claim 39, in which said at least one substitution mutation is a substitution of arginine for an amino acid selected from the group consisting of glutamic acid, aspartic acid, serine, threonine, alanine and glutamine.

5 41. The protein according to claim 39, wherein said protein contains (i) an arginine 241 to glutamic acid substitution mutation, (ii) an arginine 243 to glutamic acid substitution mutation, or (iii) both an arginine 241 to glutamic acid substitution mutation and an arginine 243 to glutamic acid substitution mutation.

10 42. The protein according to claim 39, wherein said protein contains (i) an arginine 240 to glutamic acid substitution mutation, (ii) an arginine 251 to glutamic acid substitution mutation, or (iii) both an arginine 240 to glutamic acid substitution mutation and an arginine 251 to glutamic acid substitution mutation.

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